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Review Paper

Viral safety of solvent/detergent-treated blood products

B. Horowitz, A. M. Prince, J. Hamman and C. Watklevicz

Laboratory research that began in 1982 led to the licensing in the USA of a solvent/detergent (SD)-treated factor VIII concentrate in 1985. The licence was granted on the basis of several factors. First, studies had demonstrated the inactivation of several marker viruses (vesicular stomatitis virus, Sindbis virus, Sendai virus) and other viruses such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and non-A, non-B hepatitis virus (NANBHV; now known principally to be hepatitis C virus) added to the factor VIII concentrate just before treatment. Secondly, it had been realized that the relevant viruses in transfusion (e.g. HIV, HBV, NANBHV) all had lipid envelopes. Finally, laboratory, preclinical and clinical evidence indicated that factor VIII and other proteins present in the preparation were unaffected by SD treatment. The applicability of the SD method to a wide range of products and preparations, high process recoveries and a growing body of viral safety information linked with the failure of several other virus-inactivation methods to eliminate hepatitis transmission fostered the adoption of SD technology by more than 50 organizations worldwide. SD mixtures are now used in the preparation of a diverse array of products. Numerous laboratory and clinical studies suggest that coagulation-factor concentrates and other SD-treated products prepared from plasma pools are now safer than the individual units from which they were derived. Also, a large body of evidence indicates that hepatitis A virus (HAV) is not typically transmitted by blood and blood products. Even if a rare unit contaminated with HAV was included in the plasma pool, protection is afforded by immune neutralization, other mechanisms of inactivation, and by removal. Where the possibility of HAV transmission has been reported, other factors may have contributed to its spread.

Key words: Solvent/detergent, factor VIII, haemophilia, virus inactivation, hepatitis.

Introduction

The solvent/detergent (SD) virus-inactivation method, licensed by the US Food and Drug Administration (FDA) in 1985 for use in the manufacture of a factor VIII concentrate, has gained widespread popularity in the manufacture of biological products. This popularity arises from several advantages of the treatment: its high virucidal action, high protein compatibility, ease of insertion into virtually any pre-existing or newly developed purification process, and the availability of extensive documentation, speeding both implementation and regulatory review. This review attempts to provide the historical context and an up-to-date summary of SD usage.

Basis for regulatory approval

The use of SD treatment to eliminate virus infectivity of blood protein fractions was an extension of the tradition of using ethyl ether and Tween 80 to distinguish lipid-enveloped from protein-coated viruses. In 1982, the causative agent of non-A, non-B hepatitis (NANBH) had not been isolated, although infectious serum samples were available for study. On the basis of the ether/Tween sensitivity of the Hutchinson isolate, the authors were amongst the first to demonstrate that at least this strain of non-A, non-B hepatitis virus (NANBHV) has a lipid envelope.[1] Simultaneously, they demonstrated that hepatitis B virus (HBV) is also inactivated by this method. A similar conclusion regarding NANBHV was reached using chloroform.[2] Exploration of the effect of ether/Tween treatment on selected plasma proteins indicated that their biological function was largely retained. Subsequently, because of its superior properties, tri(*n*-butyl)phosphate (TNBP) was adopted in place of ethyl ether.

The authors are with the New York Blood Center, 310 East 67th Street, New York, NY 10021, USA. Tel: (+1) 212-570-3000; Fax: (+1) 212-570-3195. Address correspondence to Dr Bernard Horowitz.

Confidence that the use of SD treatment would enhance the safety of coagulation factor concentrates came from several virus-inactivation studies. First, vesicular stomatitis virus (VSV), Sindbis virus and Sendai virus, used as markers, were all killed relatively rapidly, and to the extent of challenge ($\geq 10^5$ tissue culture infectious doses ($TCID_{50}$)), on exposure of the factor VIII concentrate to 0.3% TNBP and 0.2% sodium cholate at 30°C for 6 h. The non-enveloped encephalomyocarditis virus, used as a control, was not inactivated.[3] Secondly, on the basis of the absence of transmission of hepatitis to chimpanzees on intravenous infusion of a pool of factor VIII concentrates, which were derived from 13 different batches and five US manufacturers and treated by the authors with TNBP/cholate, they concluded that it was highly likely that all forms of blood-borne NANBHV had a lipid envelope.[4] It should be noted that the factor VIII concentrates used were not derived from donors screened by any of the screening methods introduced in 1985 or later, and were neither virally inactivated nor highly purified. Indeed, subsequent challenge of the chimpanzees with the untreated pool resulted in classic NANBH. Thirdly, they showed that $\geq 10^4$ chimpanzee infectious doses (CID_{50}) of each of HBV and hepatitis C virus (HCV) were inactivated on treatment of a factor VIII concentrate with TNBP/cholate.[4] Fourthly, following isolation and growth of human immunodeficiency virus (HIV) in long-term culture by Gallo *et al.*,[5] the authors showed that HIV was rapidly and completely inactivated to the extent of challenge on treatment of factor VIII concentrate with TNBP/cholate.[6] Importantly, the recovery of factor VIII procoagulant activity was very high following TNBP/cholate treatment and the reagents were easily removed. On the basis of the above findings, and on the normal function of the SD-treated factor VIII concentrate in a clinical setting, this procedure was first licensed by the US FDA in 1985.

Broad protein compatibility

Subsequently, a wide variety of proteins of differing structures and functions was examined, including factor VIII, factor IX, fibrinogen, fibronectin, specific IgG antibodies, haemoglobin, interferon-α, and tumour necrosis factor.[7] High protein concentration or the presence of even 2 mol glycine/l did not affect virus inactivation or protein recovery. A high lipid content, such as is found in whole plasma, could be accommodated by raising the concentration of TNBP, raising the temperature of treatment, or both. On the basis of this study, the authors concluded that SD treatment was broadly applicable. This has since been confirmed by published and unpublished clinical studies assessing circulatory recovery and/or half-life of protein preparations as diverse as intermediate purity[4] and monoclonal antibody-purified factor VIII,[9,10] factor IX,[11,12] fibrinogen, factor V, normal IgG,[13] hyperimmune IgG[14] and monoclonal IgM.

Additional pre-clinical evidence of virus safety

Since the authors' initial investigation, the number and titre of viruses used in validation studies have increased substantially (Tables 1 and 2). In each case, regardless of the enveloped virus used or challenge dose, virus inactivation was complete.

Evidence of virus safety in formal clinical trials

Numerous prospective clinical investigations to evaluate virus safety have been carried out on SD-treated products (Table 3).[15–23] In aggregate, 17.6 million units were infused, and 0/55, 0/449, and 0/524 patients developed signs of HBV, HCV and HIV transmission, respectively. It is interesting to note that one centre prepared a factor VIII concentrate by heating in the lyophilized state at 60°C for 72 h (intermediate purity) and a prothrombin-complex concentrate (PCC) using SD treatment from the same plasma pool. The SD-treated PCC proved to be safe, whereas the heated factor VIII concentrate transmitted HBV, HCV and HIV.[24] This finding is all the more remarkable as these viruses are known to concentrate away from the PCC fraction and into the factor VIII fraction during

Table 1. Virus inactivation with TNBP/detergent

Virus	Inactivation (log_{10})
VSV	≥ 9.2
Sindbis virus	≥ 8.8
Sendai virus	≥ 6.0
HBV	≥ 6.0
HCV	≥ 5.0
HIV-1	≥ 11.0
HIV-2	≥ 6.0
Duck HBV	≥ 7.3
CMV	≥ 6.0
Herpes simplex virus type-1	≥ 5.8
Ventzuels equine encephalomyocarditis	≥ 6.0
Parainfluenza 3	≥ 4.0
Murine leukaemia (Mov-3)	≥ 6.0
Murine xenotropic virus	≥ 4.0*
Rauscher murine leukaemia ecotropic virus	≥ 2.0*

* Virus was reduced to below the level of detection within minutes; at 5.5 h virus was respiked into solution and completely inactivated.

Table 2. Summary of chimpanzee studies

Test material	Treatment conditions[a]	Virus dose	Number of chimpanzees	Results (positive/total)
Pool of commercial factor VIII concentrates	1	Endogenous[b]	2	0/2
HCV added to a factor VIII concentrate	2	10^4	2	0/2
HBV added to a factor VIII concentrate	2	10^4	2	0/2
HDV added to a factor VIII concentrate	2	10^4	2	0/2
HCV added to Cohn fraction II	3	10^3	2	0/2
HCV added to plasma	4	10^3	2	0/2
HBV added to plasma	4	10^4	2	0/2
HBV added to plasma	5	10^4	2	0/2[c]
HCV added to plasma	5	10^3	2	0/2

[a] 1, 0.3% TNBP, 0.2% sodium cholate at 24°C for 6 h; 2, 0.3% TNBP, 0.2% sodium cholate at 30°C for 6 h; 3, 0.3% TNBP, 1% Tween 80 and 24°C for 6 h; 4, 2% TNBP at 37°C for 4 h; 5, 1% TNBP, 1% Triton X-100 at 30°C for 4 h.

[b] Pool of 13 batches from five US manufacturers.

[c] Chimpanzee number 362 died of an anaesthesia-related incident after 28 weeks of follow-up.

Table 3. Viral safety of SD-treated coagulation factors

Concentrate infused	Total units (approx)	Results (positive/total)			References
		HBV	NANBHV	HIV	
FVIII	145 000	na	0/17	0/18	15,16
FVIII	ua	na	0/27	0/27	17
FIX	ua	na	0/5	0/5	
FVIII	ua	na	0/165	0/49	H.-H. Brackmann, personal communication
PCC	3 848 600	0/16	0/25	0/47	A. L. Gonzaga, personal communication
FVIII	9 823 950	0/22	0/40	0/167	
FVIII	1 371 600	na	0/23	0/40	18
FVIII	1 272 000	na	na	0/29	19
FVIII	165 000	0/14	0/31	0/31	G. Mariani, personal communication
FVIII	ua	na	0/109	0/60	20
FVIII	541 000	na	na	0/18	21
PCC	265 000	na	na	0/8	
FVIII	158 600	na	na	0/6	22
FVIII	ua	0/3	0/7	0/19	23
Totals	17 590 750	0/55	0/449	0/524	

ua, unavailable; na, not applicable.

cryoprecipitation. Also, the factor VIII concentrate was subjected to little additional purification, and the PCC was chromatographically purified.

Routine clinical use

SD-treated products have been approved for routine use in numerous countries. Table 4 shows a list of products that are approved and the approximate number of doses transfused following approval. It should be noted that the 6 million doses of factor VIII transfused represent over 75 000 man-years of treatment, assuming an average infusion of 80 000 IU per man-year. On the basis of current usage patterns, approximately two out of three of the factor VIII doses transfused in North America, western Europe and Japan are SD-treated. Throughout this time period, no single case of HBV, HCV or HIV transmission has been reported. To place this in perspective, before 1985, studies in chimpanzees and clinical studies of the first of the dry-heat-treated factor VIII concentrates available in the USA showed that essentially every vial contained HCV, and transmission of HIV and HBV occurred frequently.

HIV immunoglobulin

In response to the acquired immunodeficiency syndrome (AIDS) epidemic, the authors developed procedures for the manufacture of a hyperimmune anti-HIV immunoglobulin derived from individuals infected with HIV but without overt signs of disease. Because of the potential danger to fractionation technicians, the plasma was virally inactivated with TNBP at the time of pooling. Subsequently, the IgG was isolated by Cohn–Oncley cold ethanol fractionation, and was given a second treatment with SD to further assure safety to the recipient. As a safety test, both the virally inactivated plasma and the purified final product were injected into chimpanzees. Despite being prepared exclusively from high-risk donors, neither sample resulted in the transmission of HIV or hepatitis.[14] Moreover, the injected IgG exhibited a normal circulatory recovery and half-life. In a subsequent study, the prevention of HIV infection on challenge of an injected animal with 10 CID_{50} of HIV[25] (but not 200 CID_{50}[14]) has led to the evaluation of HIV immunoglobulin prepared using SD treatment in the USA and Europe.

SD-treated plasma

At one time, the viral danger presented by coagulation-factor concentrates greatly exceeded the danger from single-donor products, for example, fresh-frozen plasma (FFP) or cryoprecipitate. With the development of powerful virus-inactivation procedures, factor VIII concentrates and other virally inactivated plasma products are now safer than non-virus-inactivated individual donations from which they were derived. This level of safety has encouraged the development of SD-treated plasma as a substitute for FFP.[26] Briefly, units of FFP are combined, thawed, treated with 1% TNBP and 1% Triton X-100 at 30°C for 4 h, the reagents removed by hydrophobic chromatography, and the final product sterile filtered, frozen, and, optionally, lyophilized. Virus inactivation has been extensively validated. Under these conditions of SD treatment, the rate of VSV and Sindbis virus inactivation exceeds that observed with factor VIII concentrates, treated either with TNBP/cholate or TNBP/Tween. The authors have also shown that $\geq 10^4$ CID_{50} of HBV, $\geq 10^5$ CID_{50} of HCV and $\geq 10^{7.2}$

Table 4. Reported usage of SD-treated products: 1985–March 1994

Product	Amount	Doses (approximate)
FVII	1.9 MU	1 900
FVIIa	2.6 MU	2 600
FVIII	6 085 MU	6 085 000
FIX	353 MU	353 000
Prothrombin-complex concentrate	113 MU	105 667
Fibrin glue	325 930 ml	65 186
Fibrinogen	93 300 g	23 300
Intravenous and intramuscular immunoglobulins	1 266 245 g	253 249
Mab IgM	2 697 units	2 697
Anti-D IgG	–	83 702
Plasma	789 479	197 400
Total dose		7 173 701

MU, million units.

$TCID_{50}$ of HIV are killed. Additionally, because of their interest in validating the duck HBV model, they have shown that $\geq 10^{7.3}$ ID_{50} of duck HBV are inactivated. Coagulation-factor content is high and in the range expected for FFP, and clinical results are excellent.[27–29]

Hepatitis A virus

The SD method does not inactivate non-enveloped viruses, and the foundation for use in a blood transfusion setting was the realization that the principal viruses of concern (HBV, all forms of NANBHV including HCV and all retroviruses including HIV) were all enveloped. A discussion of hepatitis A virus (HAV) would appear to be in order as there have been several recent reports of hepatitis A amongst recipients of a factor VIII concentrate purified by ion-exchange chromatography and SD treated.[30–32]

Transmission of HAV to blood recipients has occurred only rarely. For example, HAV transmission did not occur in any of the numerous, prospective studies of single-donor products designed to monitor hepatitis transmission conducted in the past 10–15 years (Table 5).[33–46] Moreover, HAV was not transmitted to chimpanzees in any of the evaluations assessing coagulation-factor concentrates, including the challenge phase when animals received the non-virally inactivated control. Moreover, the authors are aware of only one case of HAV reported in any of the numerous, prospective safety trials conducted with any coagulation-factor concentrate, independent of the viral-inactivation method used.[47]

Regarding the recent HAV outbreaks, the first was associated with a SD-treated factor VIII concentrate manufactured at one site in Italy from US commercial plasma. At least 41 cases of hepatitis A, widely dispersed throughout Italy, were described;[30] 38 cases (93%) involved icteric disease. Lot tracking indicated that if the SD-treated factor VIII concentrate was the vector in all cases, at least 15 different batches of the product were involved for the first 20 patients. The factor VIII production method used ion-exchange chromatography in addition to SD treatment. Between batches, the column was regenerated and sanitized with sodium hydroxide, which has been shown to be an effective sterilant for HAV.

Thirty cases (13 icteric) of HAV transmission amongst haemophilia A patients in Ireland have recently been reported.[48] Locally collected plasma was shipped to a different fractionator than described above; however, factor VIII was prepared by the same process method. Ireland was experiencing an outbreak of hepatitis A at the time the plasma was collected, and all cases could be traced to a single plasma shipment.

In Germany, 13 cases of hepatitis A amongst recipients of factor VIII concentrates have been described. The implicated factor VIII concentrate was produced by the method described above. Hepatitis A did not occur in haemophiliacs treated with other factor VIII

Table 5. Absence of hepatitis A transmission on transfusion of blood

Country	Number of patients	Units transfused	Number						Reference
			PTH	HAV	NANBH	HBV	CMV	EBV	
USA	1 528	5 564	171	0*	156	15			33
USA	283	3 359	36	0*	35				34
Netherlands	380	740	15	0*	13	0	1	1	35
Australia	842	4 789	18	0*	14	3	1		36
Sweden	74	814	15	0*	14	0	1		37
UK	248	1 796	38	0*	38	0	0		38
Spain	230	936	40	0*	29	10	1		39
Italy	246	1 500	34	0*	29	+	+		40
Israel	50	606	4	0*	4	0			41
France	64	447	5	0*	4	0	1		42
Italy	676	4 813	96	0*	92	3			43
Germany	417	2 270	16	0*	15	1			44
Sweden	742	3 342	19	0*	14	0	5		45
Italy	780	5 200	52	0*	50	0	1		46
Finland	685	8 436	11	0*	11	0	0	0	F. Ebeling, personal communication
Total	7 245	44 612	570	0	518	32	11	1	

* Specific HAV serological testing used to define HAV transmissions.
PTH, post-transfusion hepatitis; HAV, hepatitis A virus; NANBH, non-A, non-B hepatitis; CMV, cytomegalovirus; EBV, Epstein–Barr virus.

Table 6. HAV elimination during manufacture of factor VIII concentrate purified by ion-exchange chromatography

Step	Infectivity (total RFU; $\log_{10}$)			Radioactivity (total counts/min; $\log_{10}$)		
	Start	End	Difference	Start	End	Difference
Removal						
Cryoprecipitation[a]	6.49	3.86	2.63	4.16	2.06	2.09
Filtration	6.83	6.59	0.24	4.74	4.29	0.45
Ion-exchange chromatography	4.20	2.93	1.27	4.32	3.30	1.02
			Sum: 4.14			Sum: 3.56
Neutralization/inactivation						
Neutralization in cryo extract	5.75	< 3.6	> 2.15	-	-	-
Lyophilization	3.96	1.68	2.28	-	-	-
			Sum: > 4.43			
Combined[b]						
Removal	4.14					
Neutralization/Inactivation	> 4.43					
Sum:	> 8.57					

[a] Performed with HAV antibody-negative plasma.
[b] Based on HAV-infectivity determinations.
RFU, Radioimmunofocus units.

products. The first four cases occurred at the same clinic in 1988 within a 6-week period. As these patients received different factor VIII batches and as the infections occurred at least 15 months before subsequent cases, it seems reasonable to conclude that these were probably the result of community transmission. An additional complicating factor is that factor VIII used by control subjects was of much lower purity and probably contained HAV antibody. Such antibody could passively protect recipients against HAV sources in the community.

In contrast, epidemiological investigations in the USA,[49] France,[50] Brazil[51] and Norway[52] support the idea that coagulation-factor concentrates generally, and SD-treated products specifically, do not transmit HAV, at least when prepared in these countries. Moreover, 72 batches of factor VIII and factor IX concentrates examined recently for HAV gene sequences by polymerase chain reaction were all negative.[53] These results contrast with those reported by Normann *et al.*,[32] who found HAV gene sequences in two batches of factor VIII. In this case, it is interesting to note that the gene sequence of the amplified HAV product found in the factor VIII concentrate differed from that found in the patient (B. Flehmig, personal communication). This new finding supports the idea that the factor VIII concentrate may not have been the source of HAV that caused the infection.

Finally, direct evidence indicates that substantial neutralization/removal of HAV occurs during the course of manufacture of factor VIII concentrates purified using ion-exchange chromatography.[54] For these experiments, HAV endogenously labelled with [^{3}H]uridine was used. Additionally, infectivity titrations were carried out by radioimmunofocus assay in cultures of African green monkey kidney cells. As indicated in Table 6, processing resulted in the removal of 4.1 $\log_{10}$ HAV and in the neutralization/inactivation of > 4.4 $\log_{10}$, giving an overall elimination of > 8.5 $\log_{10}$ HAV. Importantly, the authors have shown that the presence of SD reagents does not interfere with antibody neutralization of added HAV. If anything, SD treatment enhanced neutralization, probably by disaggregating lipid-aggregated virus, thus enhancing its interaction with antibody.

Conclusions

The SD method of virus inactivation has been validated extensively with regard to virus inactivation and protein compatibility. SD-treated products would appear to enjoy a high margin of safety with regard to all major blood-borne viruses, including HBV, HCV and HIV. The SD method can be inserted readily into virtually any purification scheme, providing predictable and effective virus inactivation. The high specificity of the reaction derives from its mechanism of action, being directed against the lipid coat of enveloped viruses. Non-enveloped viruses, should they be present, will not be inactivated; thus, protection against these viruses needs to come from other factors, for example, donor selection, antibody neutralization, and/or complementary methods of viral removal and inactivation. Fortunately, in a transfusion setting and with products of cell culture, including monoclonal antibodies and recombinant DNA-derived products,

non-enveloped viruses present little if any risk. Several recent outbreaks of hepatitis A in haemophiliacs in Europe treated with a factor VIII concentrate prepared using the SD method and the same ion-exchange chromatographic system contrast with the absence of hepatitis A transmission in the USA, Japan, France, Brazil and Norway, and with the historical hepatitis A safety exhibited by all blood products, including coagulation-factor concentrates. Where the possibility of HAV transmission has been reported, a combination of factors may have contributed to HAV spread, including non-plasma related sources of contamination, the impact of blood collection during a community-wide HAV outbreak, and the increased transmission of HAV to haemophiliacs from community sources after the initiation of use of high-purity concentrates that lack protective anti-HAV antibodies.

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